Changes in the resistance of *Plasmodium falciparum* to chloroquine in Hainan, China

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In 1979, in view of the widespread resistance of Plasmodium falciparum *to chloroquine in the island of Hainan, China, its use as an antimalarial was suspended throughout the island. A longitudinal survey of the chloroquine-sensitivity of* P. falciparum *was carried out over the period 1981–91 to investigate whether its resistance had changed from the 1979 level.*

In-vitro *assays were carried out every 2–3 years, while* in-vivo *tests were performed annually over the period 1981–83 and also in 1991. Resistance to chloroquine declined progressively after its use had stopped. The* in-vitro *tests indicated that the rate of chloroquine-resistant* P. falciparum *was 97.9% in 1981, but dropped to 60.9% in 1991 (*P *<0.001). The mean concentration of chloroquine for complete inhibition of schizont formation was 10.4 pmol/µl in 1981, but decreased to 3.0 pmol/µl in 1991 (*P *<0.001). The proportion of samples taken from malaria cases that required high concentrations (>6.4 pmol/µl) of chloroquine for complete inhibition of schizont formation was 83.3% in 1981, but only 17.4% in 1991 (*P *<0.001); at low concentrations (<1.6 pmol/µl), the corresponding proportions increased from 4.2% in 1981 to 60.8% in 1991 (*P *<0.001). In the 4-week* in-vivo *test, the rate of chloroquine-resistant* P. falciparum *decreased from 84.2% in 1981 to 40% in 1991 (*P *<0.001). RII + RIII cases represented 59.4% of the total resistant cases in 1981, but decreased to 37.5% in 1991 (0.02 >* P *> 0.01).*

The first case of chloroquine-resistant *Plasmodium falciparum* malaria was detected in Ya County, Hainan island, in 1974. Subsequently, a large-scale survey of the resistance of *P. falciparum* to chloroquine was conducted throughout the island over the period 1975–78. The results demonstrated that such resistance was spreading rapidly and that by 1978 it was fairly extensive. The situation was most serious in south-western Hainan, where there were more chloroquine-resistant malaria cases, and the degree of resistance was generally high; the proportion of RIII malaria cases reached 30% (*1, 2*). In view of this, health officials in Hainan stipulated that chloroquine use should be stopped throughout the island beginning in 1979 and that piperaquine should replace it for malaria therapy and prophylaxis. In order to monitor any changes in the resistance of *P. falciparum* to chloroquine following the cessation of its use we conducted a longitudinal survey over the period 1981–91 in Ledong county, Hainan, where a high degree of chloroquine resistance had existed.

Materials and methods

The *in-vitro* microtest for assessing the response of *P. falciparum* to chloroquine was performed according to the standard WHO protocol (*3, 4*).[a] The microtitration plates and culture medium used in the test were prepared by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine; these preparations have the same effectiveness as those provided by WHO. The growth and chloroquine concentration required to completely inhibit schizont formation were in agreement with the results of WHO field trials, while the harvesting period used was 2–7 hours, shorter than the WHO period (6–8 hours). Growth of *P. falciparum* in wells where the level of chloroquine was >8 pmol/µl was taken to indicate resistance to chloroquine.

The WHO 28-day test was used for *in-vivo* observations (*8*). Chloroquine phosphate tablets were obtained from the Shanghai Fourteenth Pharmaceutic Factory. Falciparum malaria patients were hospitalized for 7 days; follow-up blood examinations were carried out every week for 3 consecutive weeks.

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[a] Instructions for use of the microtest kit for the assessment of the response of *Plasmodium falciparum* to chloroquine and mefloquine *in vitro*. WHO Workshop on the *in-vitro* microtest for assessment of the sensitivity of *Plasmodium falciparum* to chloroquine, Shanghai, 1979.

DDT residual spraying was performed over the period 1981–83 to prevent reinfections, but was abandoned from 1986 onwards (*9*).

The majority of the 216 study subjects to be assessed were outpatients at the county hospital and were aged 5–61 years. Most were Li ethnic minority; a few were Han or temporary workers. None had received any antimalarials, sulfonamide or sulfone drugs, according to their histories. Examination of blood samples indicated that all the study cases were infected with the single species *P. falciparum*. The density of asexual parasitaemia was in the range 1000–80 000/mm^3. "O" type red blood cells from a healthy person with no history of malaria were used to dilute the parasite density to 30 000–50 000/mm^3 in instances of high parasitaemia (*10, 11*). Haskins's method detected no traces of chloroquine and the lignin test detected no traces of sulfonamide[b] in samples of urine from any study subject. All the tests were conducted in July–September, the peak malaria transmission season in Hainan.

Results

A gradual decrease in the proportion of *P. falciparum* samples that were resistant to chloroquine (resistance rate) occurred over the study period. The results of the *in-vitro* microtest indicated that the resistance rate declined from 97.9% in 1981 to 60.9% in 1991 ($P < 0.001$) (Table 1).

The *in-vivo* test indicated that the resistance rate of *P. falciparum* to chloroquine decreased from 84.2% in 1981 to 40.0% in 1991 (Table 2).

Also, in the *in-vitro* test, *P. falciparum* samples from 83% of falciparum malaria cases exhibited complete inhibition of schizont formation at high concentrations of chloroquine (>6.4 pmol/µl) in 1981; the proportion fell to 17.4% in 1991. In contrast, samples from 4.2% of falciparum malaria cases exhibited complete inhibition of schizont formation at low chloroquine concentrations (<1.6 pmol/µl) in 1981; the proportion increased to 60.8% in 1991. Furthermore, the *in-vitro* microtest indicated that the mean concentration of chloroquine required to inhibit schizont formation fell from 10.4 pmol/µl in 1981 to 3.0 pmol/µl in 1991, a reduction of 71.2% ($P < 0.001$) (Table 3).

The results of *in-vivo* tests indicated that clearance of *P. falciparum* asexual forms in the blood of falciparum malaria cases took a mean of 72 hours in 1981, but had decreased to 59 hours in 1991. Concurrently, the proportion of patients exhibiting RII and RIII resistance fell from 59.4% in 1981 to 37.5% in 1991 ($0.02 > P > 0.01$) (Table 4).

[b] *Instructions for use of the WHO test kit for the assessment of the response of* Plasmodium falciparum *to chloroquine.* Unpublished WHO document MAP/79.1, 1979.

Table 1: **Changes in the resistance rate of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the *in-vitro* test**

	No. of cases assessed	No. of sensitive cases	No. of resistant cases	Resistance rate (%)
1981	48	1	47	97.9
1982–83	45	4	41	91.1
1986–89	77	15	62	80.5
1991	46	18	28	60.9

Table 2: **Changes in the resistance rate of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the *in-vivo* test**

	No. of cases assessed	No. of sensitive cases	No. of resistant cases	Resistance rate (%)
1981	38	6	32	84.2
1982–83	32	5	27	84.4
1986–89	20	12	8	40.0

Discussion

The resistance of *P. falciparum* to chloroquine, which has had considerable impact on malaria control, is an issue that is eliciting global concern (*12*). The possibility that the sensitivity of *P. falciparum* to chloroquine could resume after its use has been stopped for a period of years is attractive. WHO has considered such a possibility, but no clear evidence to support this hypothesis has previously appeared (*13*). Nevertheless, in Vietnam the susceptibility of *P. falciparum* to chloroquine increased after the drug's use was stopped for 10 years (*14*). Also, in Thailand, the sensitivity of *P. falciparum* to chloroquine increased markedly over the period 1978–86 after alternative antimalarials were used (*15*). Our results also document a gradual increase in the sensitivity of *P. falciparum* to chloroquine in Hainan as a consequence of the decision made in 1979 to stop using the drug following the discovery of the first case of chloroquine-resistant falciparum malaria in 1974.

Since 1979, piperaquine has been used in place of chloroquine. Almost all doctors and health workers in Hainan have treated malaria patients with piperaquine, and more recently, with piperaquine or

Table 3: **Changes in the degree of resistance of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the *in-vitro* microtest**

	No. of cases assessed	IC_{50} (pmol/µl)[a]	Mean concentration for complete inhibition of schizont formation (pmol/µl)	% of cases showing complete inhibition of schizont formation:	
				<1.6 pmol/µl	>6.4 pmol/µl
1981	48	1.3 (1.0–1.5)[b]	10.4	4.2	83.3
1982	45	0.8 (0.4–1.5)	6.1	18.2	62.2
1986–89	77	0.5 (0.3–0.9)	3.8	48.1	37.7
1991	46	0.3 (0.2–0.4)	3.0	60.8	17.4

[a] IC = inhibitory concentration.
[b] Figures in parentheses are the 95% confidence interval.

artemether. In 1986 a survey was carried out to search for unused chloroquine tablets in county hospitals and village health centres, but hardly any were found, indicating that their use had all but ceased. The resistance of *P. falciparum* to chloroquine decreased at a steady rate 12 years after the withdrawal of chloroquine; the resistance rate of *P. falciparum* to it had decreased by 37.3% in the *in-vitro* microtest and by 52.5% in the *in-vivo* test, both differences being significant ($P < 0.001$). The first indication of increasing sensitivity appeared as a decrease in the intensity of resistance; a decrease in the resistance rate occurred subsequently. Comparison of the chloroquine concentration for complete inhibition of schizont formation in the *in-vitro* microtest in 1983 with that in 1981 indicated that the difference was very significant ($P < 0.001$); in contrast, the resistance rate assessed concurrently decreased by only 7.0%, which is not significant ($P > 0.05$). *In-vivo* tests also revealed a 32.0% decline in the number of RII and RIII cases ($0.02 > P > 0.01$) over the period 1981–83, but no change in the resistance rate.

For the surveillance of chloroquine resistance and any changes that it may exhibit, the *in-vitro* microtest is more sensitive than the *in-vivo* test. A decrease in the concentration of chloroquine required for complete inhibition of schizont formation, even to a slight extent, reflects the change of resistance intensity, which preceded the decrease in resistance rate.

Although in Hainan about half the symptomatic cases of falciparum malaria could currently be cured by chloroquine, it should still not be reinstated as an antimalarial to avoid progressive development of drug resistance. Further studies are planned to determine the extent to which the sensitivity of *P. falciparum* to chloroquine can be restored.

Table 4: **Changes in the degree of resistance of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the *in-vivo* test**

	No. of cases assessed	Mean time for clearance of asexual form (hours)	Degree of resistance (%):		
			RI	RII	RIII
1981	38	72.0	40.6	6.3	53.1
1982–83	32	74.2	59.3	25.9	14.8
1991	20	58.9	62.5	12.5	25.0

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Résumé

Modifications de la résistance de *Plasmodium falciparum* à la chloroquine à Hainan, Chine

En 1979, étant donné l'ampleur de la résistance de *Plasmodium falciparum* à la chloroquine dans l'île de Hainan, il a été décidé de suspendre l'emploi de cet antipaludique dans toute l'île. Une enquête longitudinale sur la chloroquinosensibilité de *P. falciparum* a été réalisée sur la période 1981–1991 afin de rechercher une éventuelle modification de la résistance par rapport à son niveau de 1979. Des titrages *in vitro* ont été effectués tous les 2 à 3 ans, et des tests *in vivo* ont été réalisés une fois par an en 1981–1983 puis à nouveau en 1991. La résistance à la chloroquine a diminué progressivement après l'arrêt de l'utilisation de ce composé. Les tests *in vitro* ont montré que la proportion de *P. falciparum* résistant à

la chloroquine est tombée de 97,9% en 1981 à 60,9% en 1991 ($p<0,001$). La concentration moyenne de chloroquine nécessaire pour l'inhibition complète de la formation de schizontes était de 10,4 pmol/µl en 1981, et n'était plus que de 3,0 pmol/µl en 1991 ($p<0,001$). La proportion de prélèvements pris chez des malades et pour lesquels une forte concentration de chloroquine (>6,4 pmol/µl) était nécessaire pour obtenir l'inhibition complète de la formation de schizontes était de 83,3% en 1981, et de 17,4% en 1991 ($p<0,001$); pour les faibles concentrations (<1,6 pmol/µl) les proportions correspondantes étaient de 4,2% en 1981 et de 60,8% en 1991 ($p<0,001$). Dans le test *in vivo* de 4 semaines, la proportion de *P. falciparum* chloroquinorésistant passait de 84,2% en 1981 à 40% en 1991 ($p<0,001$). Les cas RII + RIII représentaient 59,4% de l'ensemble des cas résistants en 1981, contre 37,5% en 1991 ($0,02>p>0,01$). Une résurgence de la population de *P. falciparum* sensible à la chloroquine a donc eu lieu à Hainan après l'arrêt prolongé de l'utilisation de ce médicament.

References

1. **Cai Jing-xian et al.** [Investigation on the sensitivity of *Plasmodium falciparum* to chloroquine at Qianjia, Hainan Island]. *Chinese journal of preventive medicine*, 1982, **16**(1): 6–9 (in Chinese).
2. **Cai Jing-xian et al.** [Ten years' studies of malignant malaria which was resistant to chloroquine in Hainan Island]. *Chinese journal of epidemiology*, 1986, **7**(2): 92–95 (in Chinese).
3. **Rieckmann KH et al.** Drug sensitivity of *Plasmodium falciparum* — an *in vitro* microtechnique. *Lancet*, 1987, **1**: 22–23.
4. **Jensen JB, Trager W.** *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle-jar method. *Journal of parasitology*, 1977, **63**: 883–886.
5. **Liu De-quan et al.** [Preparation of the freeze-dried medium and microplate for the assessment of sensitivity of *Plasmodium falciparum* to chloroquine in the *in vitro* microtechnique]. *Journal of parasitology and parasitic diseases*, 1983, **1**: 44–48 (in Chinese).
6. **Liu De-quan et al.** [Comparison of effectiveness between two kinds of media for *in vitro* microtest of sensitivity of *Plasmodium falciparum* to chloroquine and mefloquine]. *Journal of parasitology and parasitic diseases*, 1986, **4**: 256–259 (in Chinese).
7. **Ren Dao-xing et al.** [Sensitivity of *Plasmodium falciparum* in Hainan Island to chloroquine by the *in vitro* microtechnique]. *Acta microbiologica Sinica*, 1981, **21**: 510–514 (in Chinese).
8. *Chemotherapy of malaria and resistance of antimalarials. Report of a WHO Scientific Group.* Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 529) (WHO Technical Report Series, No. 529).
9. **Liu De-quan et al.** [Preliminary survey on the sensitivity of *Plasmodium falciparum* to chloroquine in China (a review)]. *Chinese journal of internal medicine*, 1982, **21**: 643–645 (in Chinese).
10. **Liu De-quan et al.** [The extent of resistance of chloroquine-resistant falciparum malaria and the geographic distribution in China]. *Journal of parasitology and parasitic diseases*, 1986, **4**: 81–85 (in Chinese).
11. **Huang Qi-lin et al.** [A successive survey on sensitivity of *Plasmodium falciparum* patients to chloroquine by *in-vitro* microtechnique and *in-vivo* test in Hainan Island]. *Chinese journal of preventive medicine*, 1985, **19**: 220–222 (in Chinese).
12. **Payne D.** Spread of chloroquine resistance in *Plasmodium falciparum*. *Parasitology today*, 1987, **3**: 241–246.
13. **Onori E et al.** Is *Plasmodium falciparum* resistance to chloroquine reversible in absence of drug pressure? *Lancet*, 1986, **1**: 319.
14. **Jacquier P et al.** Is *Plasmodium falciparum* resistance to chloroquine reversible in absence of drug pressure? *Lancet*, 1986, **1**: 1029.
15. **Bjorkman A et al.** Drug-resistant malaria: mechanisms of development and inferences for malaria control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1990, **83**: 323–324.